The University of Chicago

THE SYNTHESIS OF CYSTINOL HYDROCHLORIDE

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE PHYSICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY
JUNE, 1943

By
HARRY KROLL

CHICAGO, ILLINOIS
1946

The University of Chicago

THE SYNTHESIS OF CYSTINOL HYDROCHLORIDE

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE PHYSICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY
JUNE, 1943

By
HARRY KROLL

CHICAGO, ILLINOIS
1946

R. UNIV.
BIBLIOTHEEK
LEIDEN

ACKNOWLEDGMENT

The writer gratefully acknowledges the aid given to him by Professor M. S. Kharasch during the course of this investigation.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41533905_0069

TABLE OF CONTENTS

Page

ACKNOWLEDGMENT. ii

INTRODUCTION 1

Physiological Aspects
Isolation Procedures
Method of Assay
Chemical Investigation
Analytical and Synthetic Investigations

THEORETICAL AND SYNTHETIC ASPECTS OF THE SYNTHESIS OF CYSTINOL HYDROCHLORIDE. 12

Attempted Reduction of Ethyl Thiazolidine-4-carboxylate Hydrochloride
Reactions Involving 2-amino-1,3-Propane Diol
Reaction with Carbon Disulfide
Acetylation and Benzoylation of 2-Amino-1,3-Propane Diol
The action of acetic anhydride
The action of benzoyl chloride
The Preparation of Halogen Derivatives
The action of hydrogen bromide on the acylated 2-amino-1,3-propane diol
The rearrangement of O,N-dibenzoyl-2-amino-1,3-propane diol
Reactions with thionyl chloride
The action of phosphorous pentachloride
The Preparation of Sulfur Derivatives

THE OXYTOCIC ACTIVITY OF CYSTINOL HYDROCHLORIDE 25

EXPERIMENTAL. 26

Thiazolidine-4-carboxylic acid hydrochloride
Ethyl thiazolidine-4-carboxylate hydrochloride
Attempted reduction of ethyl thiazolidine-4-carboxylate hydrochloride
N,N,N',N'-(tetrahydroxymethyl)-thiourea
2-thio-5-hydroxy-hexahydropyrimidine
N-acetyl-2-amino-1,3-propane diol
The N,O-diacetyl and the N,O,O'-triacetyl derivatives of 2-amino-1,3-propane diol
The reaction of N-acetyl-2-amino-1,3-propane diol with hydrogen bromide
N-acetyl-β-hydroxy-α-amino-S-propyl xanthate
N-acetyl-2-amino-1,3-propane dithiouronium dihydrobromide
N,O-dibenzoyl-2-amino-1,3-propane diol
The rearrangement of N,O-dibenzoyl-2-amino-1,3-propane diol

Bis(a-amino-β-hydroxy) propyl sulfite dihydrochloride Page
N,O-dibenzoyl-α-amino-β-hydroxy propyl chlorosulfite
N,O-dibenzoyl-α-amino-β-hydroxy propyl chloride
N,O-dibenzoyl-α-amino-β-hydroxy propyl thiouronium chloride
Bis(N-benzoyl-α-amino-β-hydroxy) propyl disulfide
Bis(N,O-dibenzoyl-α-amino-β-hydorxy) propyl disulfide
Bis(α-amino-β-hydroxy) propyl disulfide

SUMMARY. 33

INTRODUCTION

The relationship between chemical structure and physiological activity has intrigued the imagination of chemists since the advent of modern structural chemistry. In recent years, rapid progress has been made in the isolation and synthesis of biologically active compounds. However, the prediction of the activity of these compounds on the basis of their structure and configuration is still very largely a matter of guesswork. One approach to the problem of synthesizing physiologically active substances is to consider the known chemical groupings which occur in biological material and then to assemble these groupings in a molecule, the structure of which is analogous to the structures of known compounds having a similar effect. With this idea in mind, a careful study of the literature on the chemical and physiological properties of the oxytocic principle of the posterior lobe of the pituitary gland was made, and an investigation of cystinol, bis(β-amino-γ-hydroxy) propyl disulfide, was proposed. Cystinol is the amino alcohol corresponding to the amino acid cystine.

Physiological Aspects

There are several well defined pharmacological activities of posterior lobe pituitary extracts, the oxytocic, the pressor, and the diuresis-inhibiting effects. Whether these different physiological effects represent different naturally occurring chemical entities, or whether they are caused by fragmentary polypeptides broken from a labile protein during the process of preparation has not been completely determined.

The oxytocic substance, when injected intravenously, produces a general contraction of smooth muscle tissue and, more specifically, a contraction of uterine muscle. This uterus-contracting property is utilized by obstetricians in inducing labor and in facilitating parturition. It is also of use in preventing post-partum hemorrhage after the expulsion of the placenta.

The specific action of oxytocin, and the small amounts

of this material required to produce the physiological reaction, suggest that this substance is a hormone requisite for normal delivery during childbirth. Although investigators in this field almost invariably describe oxytocin as a hormone, the evidence in favor of the hormonal nature of this substance is incomplete.[1] There are however several facts which indicate that oxytocin may be a true hormone. Its action is solely on the uterine musculature; the uterine cervix is insensitive to the extract.[2] Haterius and Ferguson[3] have shown that electrical stimulation of the pituitary stalk of rabbits from two to twenty-eight hours post-partum produces uterine contractions similar to those caused by oxytocin. The contractions occur even after spinal transection, vagotomy, and severance of the splanchic nerves. These facts eliminate a nervous mechanism and indicate a humoral process. Finally, there is a very close relationship between the action of the female sex hormones and the action of oxytocin. Progesterone inhibits the effect of oxytocin whereas estrin enhances this effect by increasing uterine sensitivity. This fact is of great significance since during gestation, the corpus luteum hormone is at maximum concentration throughout the organism, and estrin is present in both urine and blood as an inactive glucuronide. These two factors favor a quiescent uterus when quiescence of this organ is most desirable. Cohen and Marrian[4] have shown that, at the onset of labor, estrin in the active form appears in the blood; thus it can act synergistically with oxytocin on the uterine musculature.

Isolation Procedures

The observation that intravenous injections of posterior pituitary extracts produce a rise in blood pressure was made in

---

[1] H. B. Van Dyke, "The Physiology and Pharmacology of the Pituitary Body," The University of Chicago Press, Chicago, 1939, p. 274.

[2] C. H. Best and N. B. Taylor, "The Physiological Basis of Medical Practice," Williams and Wilkins, Baltimore, 1939, p. 1177.

[3] Haterius and Ferguson, Am. J. Physiol. 126, 489 (1938).

[4] Cohen and Marrian, Biochem J. 30, 57 (1936).

1895 by Oliver and Schafer.[5] The existence of a uterine stimulating principle was announced several years later by Dale.[6] Although different investigators have studied the chemistry of pituitary extracts, little progress has been made, the difficulties encountered are mainly due to the scarcity of the starting material, and the ease of decomposition of the active components in the extract. Previous to 1919, several active principles were postulated,[7] but the experimental details are vague and, in the light of present day information, doubtful.

Dudley[8] showed that the oxytocic substance is more soluble in butyl alcohol than the pressor principle, and he was thus able to effect a rough separation of the two. His work was later confirmed by Draper.[9] This experimental evidence was not however accepted by Abel and his co-workers,[10] who at first maintained that the active constituent of the extracts was histamine. Later, after the histamine theory had been shown to be erroneous, they contended that the different pharmacological properties of posterior lobe pituitary extracts were due to polypeptide fragments broken from a parent protein.[11]

In 1928, Kamm and his co-workers succeeded in separating the oxytocic and pressor principles by fractionally precipitating these substances from 98 per cent acetic acid extracts of pituitary concentrates with diethyl ether and petroleum ether.[12] They obtained a fairly clear cut separation of the pressor and oxytocic principles, but their manipulations were so drastic the question whether these substances were polypeptide fragments or distinct

---

[5]Oliver and Schafer, J. Physiol. 18, 277 (1895).

[6]Dale, J. Physiol., 34, 163 (1906).

[7]Fuhner, Z. exper. Med., 1, 397 (1913).

[8]Dudley, J. Pharmacol., 14, 295 (1919).

[9]Draper, Am. J. Physiol. 80, 90 (1926).

[10]Abel and Kubota, J. Pharmacol. 13, 243 (1920).

[11]Abel and Nagayama, J. Pharamacol. 15, 347 (1920).

[12]Kamm, Aldrich, Grote, Rowe, and Bugbee, J. Am. Chem. Soc., 50, 573 (1928).

molecules remained unanswered. Stehle,[13] and Stehle and Fraser[14] by utilizing a totally different procedure, were able to prepare highly potent extracts with only slight pressor activity.

DuVigneaud and Irving[15] crushed fresh pituitary glands in a hydraulic press. The press juice was dissolved in acidified water and placed in a seventeen cell electrophoresis apparatus. The pressor and oxytocic substances were separated because the pressor principle, under the applied potential moved more rapidly toward the cathode. Biological assays showed that the press juice contained nearly all the activity of the original gland. A modified method of separation and isolation, based on the procedure described by Kamm, was used to prepare highly active oxytocic and pressor extracts from the press juice.[16]

Gallagher and Potts[17] have separated the oxytocic and pressor principles of the pituitary gland by chromatographic adsorption. Oxytocin was preferentially adsorbed on permutite and then eluted with physiological saline solution. The electrophoresis and chromatographic adsorption methods are the mildest procedures yet used to prepare the active principles in question.

Very recently, van Dyke and co-workers[18] isolated from the posterior lobes of ox pituitaries a protein containing the oxytocic, the vasopressor and the diuresis-inhibiting activities. The protein had a constant solubility, and its solutions in the ultracentrifuge proved to be homogeneous; it was therefore judged to be substantially pure. These experimental facts support Abel's contention that the true hormone of the pituitary gland is a single substance from which two or more separable principles may be isolated.

---

[13] Stehle, J. Biol. Chem., 102, 573 (1933).

[14] Stehle and Fraser, J. Pharmacol. 55, 136 (1935).

[15] Irving and Duvigneaud, J. Biol. Chem., 123, 45 (1940).

[16] Ibid., and Irving and Duvigneaud, J. Am. Chem. Soc., 62, 2080 (1940).

[17] Gallagher and Potts, J. Biol. Chem., 140, cii (1941).

[18] Van Dyke, Chow, Greef, and Rothen, J. Pharm. Exptl. Therap., 74, 190 (1942).

Method of Assay

The oxytocic activity of pituitary extracts is determined by observing the contractive effects which they exercise on isolated uterine muscle of the virgin guinea pig.[19] The contractions are registered on a moving kymograph record; they are then matched with the contractions produced by given concentrations of a standard substance. If the same solutions are assayed on different strips, the method is accurate only to 20 per cent. The uterine strips are extremely sensitive; they are subject to fatigue and variation, so that to obtain reproducible assays requires extreme care.

In recent years, oxytocic activity has been determined by measuring the transient drop in the carotid blood pressure of a fowl which has received an intravenous injection of the active extract.[20] This new method is the simpler of the two; the results obtained by it are comparable to those obtained by the older uterine strip method.

Chemical Investigation

Several investigators have studied the chemical reactions of oxytocin with various reagents. The results, although they throw some light on the chemical structure of oxytocin, are far from determining the chemical groupings which give the molecule its physiological activity. A careful study of the work done permits a few generalizations to be made and a tentative working hypothesis to be set up.

Kamm compared the dialysis rates of oxytocin and of the pressor principle with the dialysis rate of adrenalin.[21] He proposed for oxitocin a molecular weight of 600, based upon these rates. Stehle and Fraser,[22] basing their calculations on the sulfur and cystine content of oxytocin suggested a molecular weight of 1778 for this substance. The accuracy of this figure depends

---

[19] W. Burn, "Biological Standardization," Oxford University Press, 1937.

[20] J. Holtz, J. Physiol., 76, 149 (1932); Coon, Arch. Int. Pharmacodyn. 62, 79 (1939).

[21] Kamm, Science, 67, 199 (1928).

[22] Stehle and Fraser, J. Pharmacol. 55, 136 (1935).

on the purity of the sample analyzed.

Cataphoresis experiments show that the oxytocic principle migrates toward the cathode in acid solution, whereas migration ceases entirely at a pH of 8 or more.[23] This fact, assuming that there is no adsorption of the active material on basic ampholoytes, may indicate that the active substance contains no free carboxyl groups, and that it either is itself a basic compound or else is bound to one. These findings suggest that the oxytocic substance is probably an amine.[24] The ultra-violet absorption spectra of the material, however, do not indicate the presence of characteristic chromophores.[25] Acetylation inactivates oxytocin; the acetylated product is 2 per cent as active as the original substance, and is stable to alkalies.[26] This fact is interesting since posterior lobe extracts are unstable to alkalies. Freudenberg, Weiss, and Biller have shown that ketene inactivates oxytocin very rapidly; they make no mention of residual activity.[27]

Gulland[28] showed that nitrous acid reacts rapidly with oxytocin to form a substance which has 35 per cent of the original activity. A secondary and slower reaction reduces the activity to zero. The presence of a primary amino group in the oxytocic principle would explain the initial rapid reaction, although it is difficult to believe that complete replacement of an amino group would not destroy all activity. The slower inactivation is probably due to the oxidizing action of nitrous acid on the disulfide linkage present in oxytocin.[29]

The reactions of various enzymes on oxytocin have been in-

---

[23] Freeman, Gulland, and Randall, Biochem. J., 29, 2711 (1935).

[24] Kamm, Aldrich, Grote, Rowe, and Bugbee, J. Am. Chem. Soc. 50, 573 (1928).

[25] Gulland and Lucas, Biochem. J., 29, 2211 (1935).

[26] Freeman, Gulland, and Randall, Biochem. J., 29, 2711 (1935).

[27] Freudenberg, Weiss, and Biller, Z. physiol. Chem., 233, 172 (1935).

[28] Gulland, Biochem. J. 27, 1211 (1933).

[29] Lough and Lewis, J. Biol. Chem. 104, 601 (1934).

vestigated. No conclusions can be drawn from these experiments because of the doubtful purity of the enzyme preparations used. Gulland and Macrae[30] studied the inactivation of the oxytocic substance by various plant and animal proteolytic enzymes. They concluded that there was present in the different preparations an unknown enzyme which destroyed the physiological activity of the pituitary extract.

Guggenheim[31] first observed the effect of alkalies on the activity of oxytocin. The extreme ease with which dilute sodium hydroxide destroys the active substance suggested to him that oxytocin may be an O-acetyl ethanol amine, analogous to acetyl choline, which behaves similarly in the presence of weak alkalies. The inactivation of posterior lobe extracts in alkaline solution is used to distinguish the oxytocic principle from histamine. Oxytocin solutions have their maximum stability at a pH of 3 to 4.[32]

The disulfide grouping plays an important role in the physiological activity of oxytocin, the sulfur content of the most potent extracts being 3.06 per cent.[33] This figure approximates the value found for insulin. However, in insulin, the sulfur content can be entirely accounted for by cystine, whereas in oxytocin only 75 per cent of the sulfur can be shown to be present in that form.[34]

Gulland and Randall[35] undertook an intensive investigation of the oxidation-reduction system of oxytocin. They found that hydrogen sulfide reduces the activity of oxytocin solutions by 50 per cent; aeration of the solutions restores their physiological activity to its original level. Electrolytic reduction also pro-

---

[30] Gulland Macrae, Biochem. J.,27, 1237 (1933); Biochem. J., 27, 1383 (1933).

[31] Guggenheim, Biochem. Z., 65, 189 (1914).

[32] Adams, J. Biol. Chem., 30, 235 (1917).

[33] DuVigneaud, Sealock, Gifford, Kamm, and Grote, J. Biol. Chem., 100, XCIV (1933).

[34] Stehle and Fraser, J. Pharmacol., 55, 136 (1935).

[35] Gulland and Randall, Biochem. J., 29, 378 (1935).

duces a 50 per cent decrease in activity; oxidation of the reduced material restores only a part of its original activity. Hence the electrolysis probably destroys a portion of the active principle. These same investigators, by submitting oxytocin solutions to the action of indicators with known oxidation-reduction potentials, and by assaying the resulting solutions, found evidence for the existence of at least one oxidation-reduction system, and for the probable existence of two.

DuVigneaud and co-workers[36] have shown that insulin may be completely reduced and inactivated by the addition of mildly alkaline solutions of cysteine and glutathione. These two reagents are supposedly specific for the redution of disulfide linkages.[37] Applying the same technique to oxytocin solutions, it was found[38] that the reduced solutions retained their original activity. Mild oxidation also produced no loss in physiological potency. In the work cited, the assays were performed by following the drop in the carotid blood pressure of a fowl subjected to intravenous injection of the solution tested. To prove that reduction had taken place, the reduced solutions were submitted to mild benzylation and methylation. When thus treated, they lost their activity, whereas the activity of the oxidized form was not affected by similar treatment. These authors concluded that the oxidized and reduced forms of oxytocin are equally active, and that the disulfide linkage is an inherent part of the oxytocin molecule. This work allows a clean cut distinction to be made between insulin and oxytocin. Insulin is a large complex molecule with a definite configuration which apparently depends on the presence of several disulfide linkages in the molecule. Reduction of the disulfide bonds produces configurational changes accompanied by loss of physiological activity. Oxytocin on the other hand is probably a relatively simple molecule with one disulfide linkage; its physiological activity is largely independent of its configuration.

Investigations have been made on the reactions of posterior

---

[36] DuVigneaud, Fitch, Rekarek, and Lockwood, J. Biol. Chem., 94, 233 (1931).

[37] Mirsky and Anson, Proc. Exper. Med. Biol., 28, 170 (1930).

[38] Sealock and DuVigneaud, J. Pharmacol., 54, 433 (1935).

lobe extracts with several reagents specific for the disulfide linkage. Sullivan and Smith[39] have shown that the rate of precipitation of lead sulfide from alkaline oxytocic solutions is roughly proportional to their physiological activities. Greenstein[40] has prepared peptides containing cystine as the central amino acid, and has shown that the addition of glycine to such peptides weaken the disulfide linkage and facilitates the decomposition of the product by alkaline solutions of lead acetate. Cystine does not undergo desulfurization under similar conditions. Fruton and Clarke[41] showed that the introduction of N-acyl groups into cystine labilizes the sulfur toward the action of alkalies. The ease with which the disulfide linkages undergoes hydrolysis is thus dependent on the pH of the reaction medium and the structure of the molecule.[42]

Sodium sulfite and sodium cyanide inactivate oxytocin irreversibly, probably by direct action on the disulfide linkage.[43] Hydrogen peroxide in alkaline solution destroys the active substance, but the inactivation is complicated by a period of partial recovery followed by irreversible inactivation. Halogens inactivate the oxytocic principles completely.

Analytical and Synthetic Investigations

Various efforts have been made to isolate definite chemical entities from posterior lobe extracts by purification and degradation. Stehle and Trister[44] isolated arginine, proline, tyrosine, cystine, and isoleucine from oxtocin hydolysates.

The presence of histidine in these extracts has been reported by Freudenberg, Weiss, and Biller.[45] Stehle and Fraser[46]

---

[39]Sullivan and Smith, U.S. Public Health Reports, XLIII, 1334 (1928).

[40]Greenstein, J. Biol. Chem., 124, 255 (1938).

[41]Fruton and Clarke, J. Biol. Chem., 106, 667 (1934).

[42]Schoberl, Ann. 534, 210 (1938).

[43]Gulland and Randall, Biochem. J.,29, 391 (1935).

[44]Stehle and Trister, J. Pharmacol., 65, 343 (1939).

[45]Freudenberg, Weiss, and Biller, Z. Physiol. Chem., 233, 172 (1935).

[46]Stehle and Fraser, J. Pharmacol.,55, 136 (1935).

failed to obtain a test for histidine in their most active extracts. The relation of these amino acids to the physiological activity of oxytocin is not known, since it is impossible to determine whether they are parts of the active molecule or whether they are constituents of extraneous peptides accompanying the active substance.

Freudenberg and Biller's[47] isolation of one per cent choline (as the reinekate) from hydolysates of oxytocin, has stimulated various workers to synthesize simple molecules to be examined for oxytocic properties. Since choline occurs rather abundantly in nearly all tissue,[48] it is exceedingly difficult to appraise the contribution of Freudenberg and Biller. The known activity of acetyl choline on the isolated uterus[49] suggested that the amino acid esters of choline may possess uterus stimulating activity. Previous to the isolation of choline with pituitary extract, Dudley[50] had prepared glycyl choline and found it to be inactive. Freudenberg and Keller[51] prepared the choline esters of glycine, dl-alanine, dl-leucine, dl-valine, but these substances were found to be devoid of oxytocic activity.

Recently, Gulland, Partridge, and Randall[52] have prepared a series of amino acyl derivatives of choline. Glycyl glycyl choline, phenyl alanyl choline, and di-thioglycollyl choline have no effect on the isolated uterus of the virgin guinea pig. Relatively concentrated solutions of cysteinyl choline and its disulfide produce only weak contractions. Dimethyl glycyl choline is devoid of oxytocic activity.[53]

The widespread occurrence of choline and amino alcohols and their derivatives indicates the biological importance of these

---

[47] Freudenberg and Biller, Naturwiss., 24, 523 (1936).

[48] Ibid.

[49] Guggenheim, M., "Die Biogenen Amine," S. Karger, Basel, 1940, p. 76.

[50] Dudley, J. Chem. Soc.,119, 1256 (1921).

[51] Freudenberg and Keller, Ber., 71, 329 (1938).

[52] Gulland, Partridge, and Randall, J. Chem. Soc., 419 (1940).

[53] Work, J. Chem. Soc., 190 (1940).

substances. Guggenheim[54] and Fuhner[55] suggested that the active substances in posterior lobe extracts were of choline-like character. Karrer and his collaborators[56] believe that amino alcohols and choline derivatives are probably derived biologically from amino acids. They reduced a group of N-acetyl amino acid esters with sodium and butyl alcohol; from the amino alcohols thus obtained, they prepared the corresponding quaternary ammonium salts. Leucine, alanine, phenyl alanine, and tyrosine esters were thus reduced and subjected to complete methylation. The compounds obtained and their acetylated derivatives all act similarly to choline and acetyl choline in that they produce increased tone in the para-sympathetic innervated organs. However, acetyl choline produces these effects at much lower concentrations.

The observed chemical properties of oxytocin permit a few generalizations to be made regarding the nature of this substance. At the present time, there is no evidence that the physiological action of oxytocin is due to any special grouping of amino acids or to the presence of a prosthetic group attached to an accompanying peptide. Following the suggestion that compounds with oxytocic activity should be synthesized from materials with a possible biological origin, cystinol, the amino alcohol analogue of cystine, should be an interesting compound for pharmacological examination in any biological process where the disulfide-sulfhydryl equilibrium plays an important role.

---

[54] Guggenheim, Biochem. Z., 65, 189 (1914).

[55] Fuhner, Biochem. Z., 75, 232 (1916).

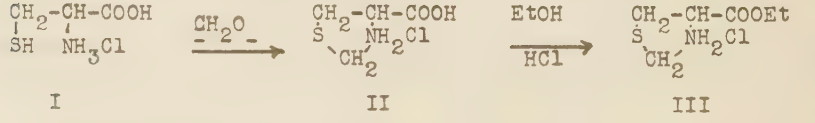
[56] Karrer, Horlacher, Locher, Thoman, and Mader, Helv. Chim. Acta., 5, 469 (1922); ibid., 4, 76 (1921).

THEORETICAL AND SYNTHETIC ASPECTS OF THE SYNTHESIS OF CYSTINOL HYDROCHLORIDE

Two possible syntheses of cystinol have been investigated. The first involved the reduction of the natural amino acid, cystine; in the second, 2-amino-1, 3-propane diol was used as the starting material, and one of the hydroxyl groups was replaced by a sulfhydryl group.

Attempted Reduction of Ethyl Thiazolidine-4-Carboxylate Hydrochloride (III)

Amino acid esters have been converted to the corresponding amino alcohols by high pressure, high temperature hydrogenation,[57] and by reduction with sodium and ethyl or butyl alcohol.[58] The first of these methods cannot be used with cystine or cysteine, and the instability of these two amino acids towards alkalies emphasizes the necessity of adopting a reduction procedure which minimizes the destructive effect of alkaline reagents. Rattner and Clarke[59] have shown that cysteine hydrochloride reacts with formaldehyde in the presence of pyridine to form thiazolidine-4-carboxylic acid hydrochloride (II), a compound which is fairly stable to both acids and alkalies. When oxidized with iodine, it is quantitatively converted to cystine. The following reactions were therefore proposed.



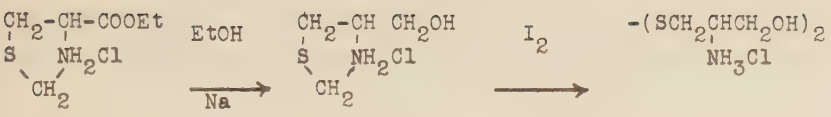
I II III

---

[57] Christman and Levene, J. Biol. Chem., 125, 709 (1938).

[58] Karrer, Horlacher, Locher, Thoman, and Mader, Helv. Chim. Acta., 5, 469 (1922); ibid., 4, 76 (1921).

[59] Rattner and Clarke, J. Am. Chem. Soc., 59, 200 (1937).

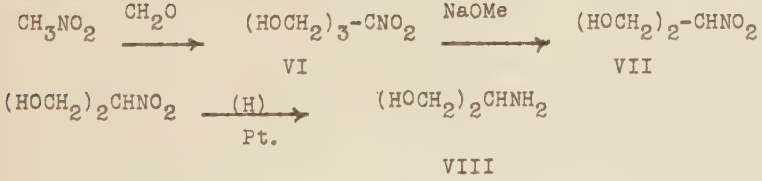


III IV V

All attempts to reduce ethyl thiazolidine-4-carboxylate (III) by ethyl alcohol and sodium were however unsuccessful. The isolation of an unidentified oil and elementary sulfur from the reaction mixture indicates that desulfurization is a predominant part of the reaction. In several of the attempted reductions glacial acetic acid was added to the reaction mixture in order to keep down the concentration of sodium ethylate.

Reactions Involving 2-Amino-1, 3-Propane Diol (VIII)

Dihydroxy isopropyl amine (2-amino-1, 3-propane diol) (VIII) is a hygroscopic solid first prepared (as a hydrochloride)[60] by the reduction of the oxime of dihydroxy acetone. A more elegant synthesis is described by Wilkendorf and Schmidt.[61] They condensed formaldehyde and nitromethane to obtain the completely hydroxylated nitro derivative (VI). This compound was submitted to the action of sodium methylate which reduced the tris-hydroxylated nitro derivative (VI) to the sodium salt of 2-nitro-1, 3-propane diol (VII). Catalytic reduction gave an almost quantitative yield of the amino diol (VIII), which was isolated as the oxalate.



VI VII VIII

Reaction With Carbon Disulfide

Knorr and Rossler[62] had previously shown that ethanol amine

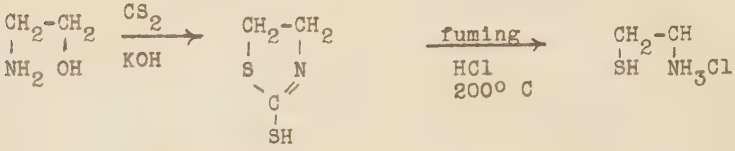
---

[60] Piloty and Ruff, Ber., 30, 1656 (1897).

[61] Schmidt and Wilkendorf, Ber., 52, 389 (1919).

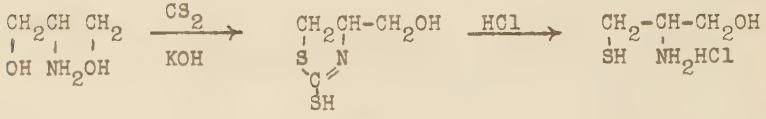
[62] Knorr and Rossler, Ber., 36, 1281 (1903).

reacts with carbon disulfide to give 2-mercapto thiazolidine (IX). This compound may be hydrolyzed with fuming hydrochloric acid at 200° C to β-amino ethyl mercaptan hydrochloride (X).[63]



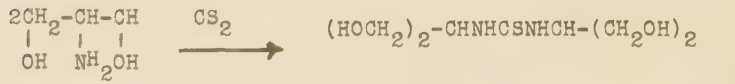
IX X

Accordingly, the following synthesis of cystinol was proposed.



XI XII

The reaction of 2-amino-1, 3-propane diol with carbondisulfide, when carried out under the conditions prescribed by Knorr and Rossler resulted in an oily residue soluble in hot water. Analysis of a purified sample showed the presence of two nitrogen atoms per atom of sulfur, which indicated that the amino diol had reacted with carbon disulfide to give the thiourea derivative (XIII).[64]



XIII

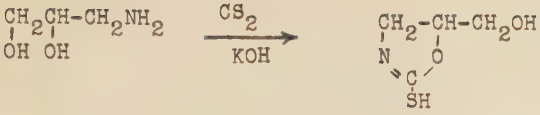
Concerning this reaction, it is interesting to note that Roux,[65] in a study of the reaction of carbon disulfide on polyhydroxy amines, obtained the corresponding oxazolidine derivative. Using 1-amino-2, 3-propane diol, he obtained 2-mercapto oxazolidine 4-methyl alcohol (XIV).

---

[63] Gabriel and Leopold, Ber., 36, 1281 (1898).

[64] Fry and Culp, Rec. Trav. Chem., 52, 1061 (1933).

[65] Roux, Ann. Chimie (8) 1, 72 (1904).



XIV

Roux explained the difference between the formation of the oxazolidine XIV instead of the thiazoldine IX as being due to the presence of the adjacent hydroxyl group; but he gave no details as to how this group might influence the reaction mechanism.

A similar reaction was here tried with 2-hydroxy-1, 3-diamino propane, and an almost quantitative yield of 2-thio-4-hydroxy hexahydropyrimidine (XV) was obtained. Similar reactions between diamines and carbon disulfide have been observed by Strack.[66]

XV

Acetylation and Benzoylation of 2-Amino-1, 3-Propane Diol

It was decided to make further attempts to prepare cystinol by replacing one of the hydroxyl groups in 2-amino-1, 3-propane diol with a halogen atom, and then replacing the halogen atom with a sulfhydryl group. In order to carry out such a procedure, it was desirable to block the two functional groups not involved in the reactions indicated. The acetylation and benzoylation of 2-amino-1, 3-propane diol were therefore investigated. The object was to prepare various acyl derivatives which could be easily purified and identified.

The action of Acetic Anhydride.--The acetylations with acetic anhydride were carried out in pyridine at room temperature. One mole of acetic anhydride reacted with one mole of the amine to give a good yield of the N-acetyl derivative (XVI).

XVI

---

[66] Strack, Z. physiol. Chemie., 180, 198 (1920).

A similar procedure in which two moles of acetic anhydride to one mole of amino diol were used resulted in a red oil from which it was difficult to remove the last traces of pyridine. The separation of the oil into identifiable pure substances was exceedingly troublesome. The N,O-diacetyl derivative (XVII) was obtained from the reaction product by vacuum distillation. The triacetyl derivative (XVIII) was obtained by repeated crystallization of the residue from acetone-ether

$$(HOCH_2)_2CHNH_2 \xrightarrow[(CH_3CO)_2O]{\text{2 mols}} CH_3COOCH_2\underset{\underset{COCH_3}{|}}{\overset{}{CH}}CH_2OH \text{ and } (AcOCH_2)_2CHNHAC$$

XVII XVIII

The exact proportions in which these compounds were formed was not determined because of the losses involved in the separation of the two products.

The action of Benzoyl Chloride.--The mono- and tribenzoyl derivatives of 2-amino 1, 3-propane diol had been prepared by Wilkendorf and Schmidt.[67] Since the dibenzoyl derivative was desired as an intermediate, the action of two moles of benzoyl chloride on one mole of amino diol in pyridine at 0° C was investigated. As in the acetylation reaction, both the di- and the triacyl derivatives (XIX and XX) were formed.

$$(HOCH_2)_2CHNH_2 \xrightarrow[\text{2 mols}]{BzCl} BzOCH_2\underset{\underset{Bz}{|}}{\overset{}{CH}}CH_2OH + (BzOCH_2)_2CHNHBz$$

XIX XX

The Preparation of Halogen Derivatives

The displacement by a halogen atom of a hydroxyl group beta to a carbon atom carrying a negative group (such as OH, NH_2, or halogen) is more difficult than the formation of a simple alkyl halide. The preparation of β-bromethyl amine hydrobromide from β-ethanol amine involves the drastic action of hydrobromic acid on the alcohol and the continuous removal of water.[68] Gabriel

---

[67] Schmidt and Wilkendorf, Ber., 52, 389 (1919).

[68] Cortese, Organic Synthesis, 18, 13 (1938).

and Colman[69] using fuming hydrochloric or hydrobromic acid were unable to replace the hydroxyl group of β-phenyl-β-amino ethyl alcohol by halogen. Similarly, Meisenheimer and Chou[70] using concentrated hydrochloric acid, could not obtain 2,2-diphenyl-2-chloro-1-amino ethane from 2,2-diphenyl-2-hydroxy-1-amino ethane. This fact is interesting in view of the extreme ease with which tertiary alcohols react with hydrochloric acid.

The mechanism suggested for the formation of alkyl halides from alcohols is as follows.

$$ROH + H_3O^+ \longleftrightarrow (ROH_2)^+ + H_2O$$

$$(ROH_2)^+ + Cl^- \longrightarrow RCl + H_2O$$

In amino alcohols, the proton would react with the more strongly basic nitrogen.

$$H_2NCH_2CH_2OH + H_3O^+ \longrightarrow [H_3NCH_2CH_2OH]^+ + H_2O$$

The second step, the addition of the proton to the hydroxyl group

$$[H_3NCH_2CH_2OH]^+ + H_3O^+ \longrightarrow [H_3NCH_2CH_2\overset{+}{O}H_2]^{++} + H_2O$$

would be made extremely difficult by the proximity of the positively charged nitrogen.

The action of hydrogen bromide on the acylated 2-amino-1, propane diol.--Since the direct action of gaseous hydrogen bromide on a dioxan solution of the 2-amino-1,3-propane diol produced only the hydrobromide of the original compound, the reaction of hydrogen bromide on the acetylated and the benzoylated derivatives of the amine diol was investigated.

A dioxan solution of the more acetylated derivative (XVI) was treated with gaseous hydrogen bromide until the calculated amount of acid was taken up. High vacuum (10^{-5} mm.) distillation of the reaction product gave a thick oily distillate which, by analysis, appeared to be a mixture of the monobromo and dibromo derivatives. Attempts to separate this oil into chemically pure constituents were partially successful. But since the high vacuum distillation caused extensive decomposition of the reaction product the whole procedure was not practical as a synthetic method. Several reactions carried out on the oil distillate corroborated the

---

[69] Gabriel and Colman, Ber., 47, 1866 (1914).

[70] Meisenheimer and Chou, Ann., 539, 70 (1939).

conclusion that the material was a mixture of the mono- and dibromo derivatives. In one experiment potassium ethyl xanthate reacted with the oil to give a small amount of ethyl (N-acetyl-α-amino β-hydroxy) propyl xanthate (XXII).

$$HOCH_2\underset{\underset{NHAc}{|}}{CH}-CH_2Br + KS-CS-OEt \longrightarrow HOCH_2\underset{\underset{NHAc}{|}}{CH}CH_2S-CS-OEt + KBr$$

XXI XXII

In a second reaction, the same oil was refluxed with thiourea in n-propyl alcohol. A fair yield of the N-acetyl-2-amino-1,3-propane dithiouronium dibromide (XXIV) was obtained.

$$AcNHCH(CH_2Br)_2 + NH_2-CS-NH_2 \longrightarrow AcNHCH(CH_2-S\overset{\overset{NH}{\|}}{C}-NH_2\cdot HBr)_2$$

XXIII XXIV

The rearrangement of O,N-dibenzoyl-2-amino 1,3-propane diol to O,O'-dibenzoyl 2-amino 1,3-propane diol hydrobromide.--The reaction of hydrogen bromide on a dioxane solution of the N,O-dibenzoyl derivative (XIX) resulted in a shift of the N-benzoyl group to the oxygen of the hydroxyl group; the rearranged compound was isolated as the amine hydrobromide (XXV).

$$BzOCH_2-\underset{\underset{NHBz}{|}}{CH}-CH_2OH + HBr \longrightarrow (BzOCH_2)_2-CHNH_2HBr$$

XIX XXV

The migration of a benzoyl group from nitrogen to oxygen has been reported by Wolfheim[71] and has been observed in a number of beta amino alcohols. The shift is a reversible one;[72] in alkaline solutions, the amine hydrobromide rearranges to the N-benzoyl derivative.[73] Bergman and Brand[74] investigated this rearrangement in the N,O-dibenzoyl derivative of 2,3-dihydroxy 1-amino propane (XXVI). By treating this compound with phosphorous trichloride or thionyl chloride in chloroform, they isolated an oxazolidine derivative (XXVII) which, when dissolved in hydrochloric acid, was

---

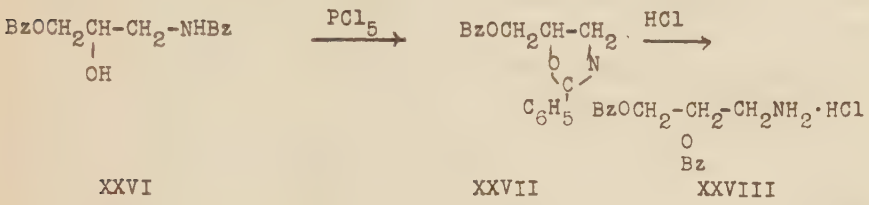
[71]Wolfheim, Ber., 47, 1441 (1914).

[72]Immediata and Day, J. Org. Chem., 5, 512 (1940).

[73]Hartung and Munch, J. Am. Chem. Soc., 53, 1875, (1931).

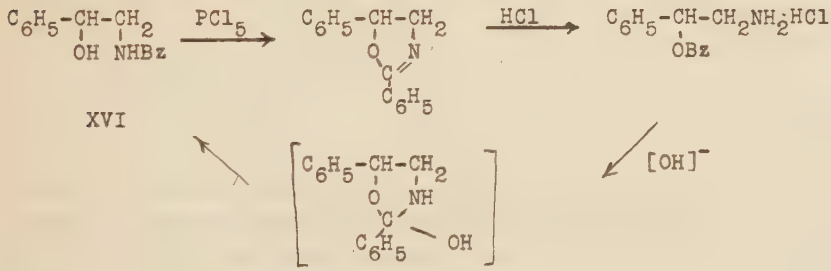
[74]Bergmann and Brand, Ber., 56, 1280 (1923).

hydrolyzed to the O,C'-dibenzoyl amine hydrochloride (XXVIII).



XXVI XXVII XXVIII

Oxazolidine formation had been previously observed by Wolfheim[75] in the rearrangement of β-phenyl-β-hydroxy-ethyl benzamide. The isolation of such heterocyclic compounds and the ease with which they are hydrolyzed to O-benzoyl derivatives has been used as evidence to support the hypothesis that they occur as the intermediates in the rearrangement of the N-acyl to the O-acyl compound. This hypothesis, however, is difficult to reconcile with several facts. Bergmann and Brand's oxazolidine derivative is stable in alkaline solution; yet no trace of this compound could be isolated in the reverse rearrangement (XXVIII ⟶ XXVI) which occurs in alkaline media. In recognition of these facts, Betzieche[76] postulated the following reaction scheme.



XVI

(Not isolated)

Immediata and Day,[77] investigating the rearrangement of the benzoyl derivatives of β-naphthyl βethanol amine, found that alcoholic hydrogen chloride produce the N⟶O benzoyl shift without

---

[75] Wolfheim, Ber., 47, 1441 (1914).

[76] Betzieche, Z. physiol. Chemie, 146, 229 (1925).

[77] Immediata and Day, J. Org. Chem., 5, 512 (1940).

intermediate oxazolidine formation. Probably phosphorous pentachloride or thionyl chloride was responsible for the dehydration and ring formation as observed by Wenker.[78] Immediata and Day also found that secondary amino alcohols undergo a similar N acyl ⟶ O-acyl shift. In these instances an oxazolidine intermediate is impossible because N-monoalkyl amino ethanols cannot form such compounds. A more probable mechanism would be one in which a common intermediate for both the N⟶O and the O⟶N shifts is postulated. The transformation of this intermediate into the O-acyl or the N-acyl derivative would then depend on the pH of the medium.

Reactions with thionyl chloride.--Thionyl chloride acts to replace groups with chlorine atoms under relatively mild conditions. A basic catalyst such as pyridine may or may not be required. Substitution of chlorine atoms for alcoholic hydroxyl groups by the action of excess thionyl chloride on a hydroxy-amine hydrochloride has been reported by several investigators.[79] Since in the present program it was desired to replace one hydroxy group in 2-amino-1,3-propane diol by a chlorine atom the reaction of one mole of thionyl chloride and one mole of the amine diol was investigated. The solid isolated was identified as the diester of sulfurous acid (XXIX).

$$2\ HOCH_2\text{-}\underset{\displaystyle NH_2\cdot HCl}{\underset{|}{CH}}\text{-}CH_2OH + SOCl_2 \longrightarrow (HOCH_2\text{-}\underset{\displaystyle NH_2HCl}{\underset{|}{CH}}\text{-}CH_2O\text{-})_2SO + 2HCl$$

XXIX

The N,O-dibenzoyl derivative XIX, when treated for a few hours with thionyl chloride in the presence of pyridine, assumed a red color. The product was a mixture of unidentifiable red oils. The same reactants, in the absence of pyridine, gave the chlorosulfite derivative (XXX).

$$BzOCH_2\text{-}\underset{\displaystyle \underset{\displaystyle Bz}{NH}}{\underset{|}{CH}}\text{-}CH_2OH + SOCl_2 \longrightarrow BzOCH_2\text{-}\underset{\displaystyle \underset{\displaystyle Bz}{NH}}{\underset{|}{CH}}\text{-}CH_2\text{-}OSOCl + HCl$$

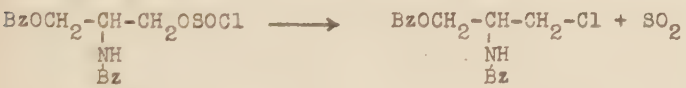
XIX XXX

---

[78] Wenker, J. Am. Chem. Soc., 57, 1079 (1935).

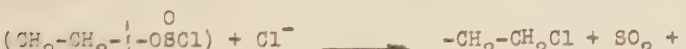
[79] Ward, J. Am. Chem. Soc., 57, 914 (1935).

Other workers have reported the isolation of chlorosulfites as intermediates in the preparation of alkyl halides.[80] Such alkyl chlorosulfites may be converted to the corresponding chlorides either by warming with pyridine or by refluxing the chlorosulfite in a suitable solvent. In the present instance, warming with pyridine proved to be an unsuitable method. Refluxing a toluene solution of XXX and then allowing the solution to stand for several days gave a small yield of N,O-dibenzoyl-β-amino-γ-hydroxy propyl chloride (XXXI).

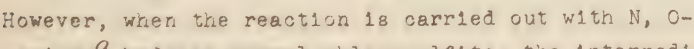


XXX XXXI

Carre[81] and his co-workers have studied the preparation and decomposition of numerous chlorosulfites and sulphurous esters of aliphatic alcohols. They found that a negative group on the beta carbon atom tends to increase the stability of the chlorosulfite. The presence of the positively charged sulfur in the chlorosulfite derivative tends to weaken the carbon-oxygen bond so that replacement of the hydroxyl group by a halogen atom is more easily effected.



The negative group on the beta carbon counteracts the inductive effect of sulfur on the carbon-oxygen bond and stabilizes this linkage. Thus the remarkable stability of the benzoylated amino-hydroxy-propyl chlorosulfite is explained. Pyridine probably forms with the chlorosulfite an unstable salt which usually breaks down to the normal products.



However, when the reaction is carried out with N, O-dibenzoyl-α-amino-β-hydroxy propyl chlorosulfite, the intermediate pyridinium salt is probably sufficiently stable to resist decomposition.

---

[80] Carre, Bull. Soc. Chim., France (5), 3, 1064 (1936).

[81] Ibid.

The action of phosphorous pentachloride on amino alcohols. --In certain instances where halogen acids or thionyl chloride fail to replace the hydroxyl group in an amino alcohol with a halogenation, phosphorous pentachloride may be used to carry out this replacement. Consequently the dibenzoyl derivative of 2-amino-1, 3-propane diol (XIV) was treated with a chloroform solution of phosphorous pentachloride. Removal of the solvent left behind an oil which solidified when treated with a mixture of chloroform and ligroin. The solid which contained both phosphorous and chlorine, slowly decomposed at room temperature to the initial di-benzoylated amino diol. This solid, judging from the stability of the chlorosulfite (XXX), is probably a chlorophosphoric ester of the di-benzoylated amino alcohol (XXXII).

$$BzOCH_2\text{-}\underset{\underset{NHBz}{|}}{CH}\text{-}CH_2OH + PCl_5 \longrightarrow BzOCH_2\text{-}\underset{\underset{N^HBz}{|}}{CH}\text{-}CH_2OPCl_4 + HCl$$

XIX XXXII

A compound similar to XXXII has been prepared by the action of phosphorous oxychloride on the N, O-dibenzoyl-2,3-dihydroxy -1-amino propane.[82] Walker and Johnson[83] in an investigation of the action of one mole of phosphorous trichloride on one mole of alcohol decided that since, scarcely any alkyl chloride was formed, the primary reaction was the formation of the chlorophosphorous ester. Gerrard,[84] in a study of the reactions of the phosphorous chlorides on butyl alcohol, came to the conclusion that the formation of n-butyl chloride was essentially due to the action of hydrogen chloride on the chlorophosphoric ester. This investigator proposed the following reaction.

$$ROH \xrightarrow{PCl_5} ROPCl_4 \xrightarrow{HCl} RCl + POCl_3 + HCl$$

Accordingly, in the work here described, the reaction of phosphorous pentachloride on the benzoylated amino alcohol was modified. After the reaction mixture had been refluxed, the solvent and ex-

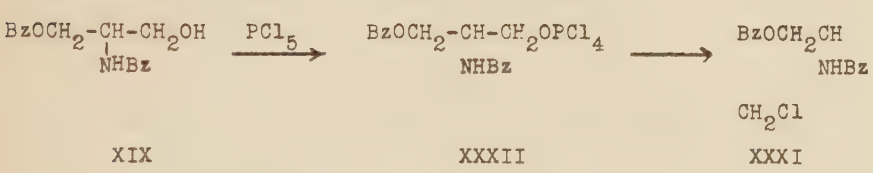
---

[82] Bergmann and Brand, Ber., 56, 1280 (1923).

[83] Walker and Johnson, J. Chem. Soc., 87, 1592 (1905).

[84] Gerrard, J. Chem. Soc., 1464 (1940).

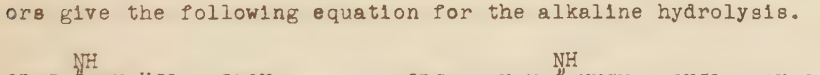
cess phosphorous pentachloride were removed under reduced pressure. The oily residue was taken up in chloroform, and dry hydrogen chloride was passed through the solution. Subsequent manipulations yielded a solid identical with N,C-dibenzoyl-β-amino-γ-hydroxy propyl chloride obtained from the thionyl chloride reaction.



XIX XXXII XXXI

The Preparation of Sulfur Derivatives

Of the various methods for the preparation of sulfhydryl compounds, the reaction of alkyl halides with thiourea and the alkaline hydrolysis of the S-substituted thiourea to the corresponding mercaptan seemed best suited for the purposes of the present investigation. This method has been extensively used for the preparation of mercaptans. Arndt[85] prepared large amounts of methyl mercaptan by this procedure. Renshaw, Driesback, Ziff, and Green[86] have prepared thiocholines and Backer and Dijkstra[87] have syntheiszed alkyl mercaptans by the same method. These investigators give the following equation for the alkaline hydrolysis.



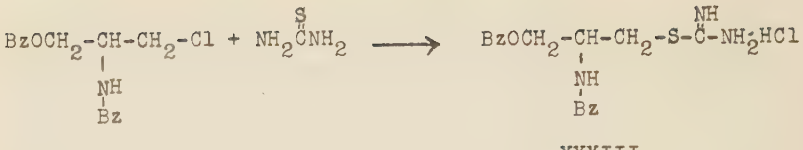
The chlorine atom in N,C-dibenzoyl-β-amino-γ-hydroxy propyl chloride (XXXI) was replaced with a sulphydryl group by refluxing the chloride with the calculated amount of thiorea in n-propyl alcohol. The amount of thiouronium chloride (XXXIII) isolated was 60 per cent of that calculated.

---

[85] Arndt, Ber., 54, 2236 (1921).

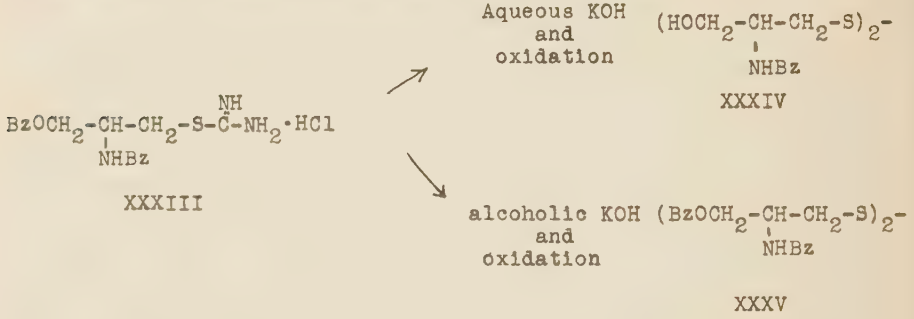
[86] Renshaw, Driesbach , Ziff, and Green, J. Am. Chem. Soc., 60, 1765 (1938).

[87] Backer and Dijkstra, Rec. Trav. Chim., 51, 289 (1935).



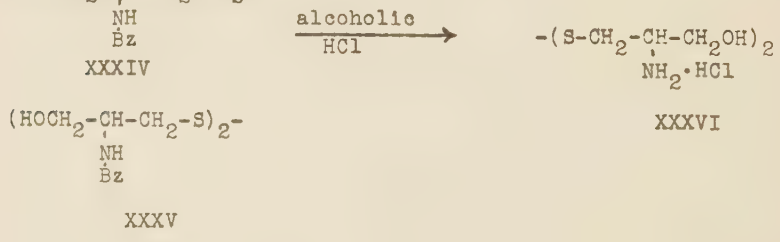
XXXI XXXIII

In the hydrolysis of the N,O-dibenzoyl-α-amino-β-hydroxy propyl thiouronium chloride (XXXIII) to the mercaptan, aqueous potassium hydroxide also removed the O-benzoyl group. Oxidation of the product by air in the presence of ferric chloride produced the bis (N-benzoyl-α-amino-β-hydroxy) propyl disulfide (XXXIV). Alcoholic potassium hydroxide on the other hand left the two benzoyl groups intact. Oxidation of the mercaptan thus obtained gave the bis (N,O-dibenzoyl-α-amino β-hydroxy) propyl disulfide (XXXV).



XXXIII XXXIV XXXV

The benzoyl groups of the mono- and dibenzoyl derivatives of bis (α-amino-β-hydroxy) propyl disulfide (XXXIV and XXXV) were removed by alcoholysis in ethanolic hydrogen chloride; the product was the desired cystinol hydrochloride (XXXVI).



XXXIV XXXVI

XXXV

THE OXYTOCIC ACTIVITIES OF CYSTINOL HYDROCHLORIDE AND RELATED COMPOUNDS

The oxytocic activities of some of the compounds previously described were determined by measuring the contractions of the isolated uterus of a virgin guinea pig when the uterine strip was exposed to a solution of the compound to be assayed. The method used was a modification of the one described by Burn.[88] Of the compounds assayed, bis(N-benzoyl-α-amino-β-hydroxy) propyl disulfide and N,O-dibenzoyl-α-amino-β-hydroxy propyl thiouronium chloride were inactive. Cystinol hydrochloride (XXXVI) at concentrations of 1 part in 85,000, caused weak uterine contractions.

---

[88] Burn, W., "Biological Standardization," Oxford University Press, 1937.

EXPERIMENTAL

Thiazolidine-4-carboxylic acid hydrochloride.--This compound was prepared by the method of Rattner and Clarke.[89]

Ethyl thiazolidine-4-carboxylate hydrochloride.--One hundred cubic centimeters of absolute alcohol and 9.5 grams of thiazolidine-4-carboxylic acid hydrochloride were placed in a 200 ml. round bottom flask equipped with a condenser and gas inlet tube. Hydrogen chloride was bubbled into the alcohol solution for twenty minutes. The solution was refluxed on a water bath for two hours while hydrogen chloride was passed into the reaction mixture. After two more hours of refluxing, the solution was cooled and the excess alcohol removed by distillation under reduced pressure. When the solid residue was crystallized from ethyl alcohol, there was obtained 6.7 grams of the ethyl ester which melted at 168-70°C. Analysis. Calcd. for $C_6H_{12}O_2NSCl$: N, 7.23; Found: N, 7.42.

Attempted reduction of ethyl thiazolidine-4-carboxylate hydrochloride.--The ethyl ester of thiazolidine-4-carboxylic acid hydrochloride, 4.2 grams was suspended in 50 ml. of absolute ethyl alcohol and the mixture was placed in a 200 ml. three necked flask equipped with a mercury sealed stirrer, condenser, and dropping funnel; the flask was immersed in an ice bath. Sodium, (3.0 gr.) was added slowly over a period of 30 minutes. After the completion of the reaction, 7.3 ml. of glacial acetic acid was added. The odor of hydrogen sulfide was apparent at this point. The alcohol solution was filtered from the precipitated sodium acetate. When the filtrate was allowed to stand, rhombic sulfur was deposited. This sulphur was separated by filtration and the solvent removed from the filtrate by distillation. An oily residue remained. No pure compound could be isolated from this oil.

N,N,N',N'-(tetrahydroxymethyl)-thiourea.--Carbon disulphide (12 gr.), of 2-amino-1,3-propane diol (9.1 gr.), ethyl alcohol (8 gr.) and potassium hydroxide (16 gr.) were thoroughly mixed and

---

[89] Rattner and Clarke, J. Am. Chem. Soc., 59, 200 (1937).

and heated on a steam bath for eight hours. The mixture was then diluted with an equal volume of water and acidified with dilute hydrochloric acid. The solution was heated to boiling, filtered, and placed in the icebox overnight. The aqueous solution was decanted from the precipitated oil which was then dried for forty-eight hours in a vacuum dessicator over phosphorous pentoxide. Analysis: Calcd. for $C_{17}H_{16}O_4N_2S$: N, 12.49; Found: N, 12.64.

2-thio-5-hydroxy-hexahydropyrimidine.--1,3-diamino-2-hydroxy propane, 20 grams, was refluxed for eight hours in a solution consisting of 20 grams of 95 per cent alcohol, 7 grams of potassium hydroxide, and 30 grams of carbon disulfide. The onset of the reaction was marked by the formation of a white precipitate and the evolution of heat. After the reaction mixture had been cooled, the supernatant liquid was decanted from the solid residue. When this solid was recrystallized from water, there was obtained 14.7 grams of needle-like crystals which melted at 186-90°C. Analysis: Calcd. for $C_4H_8ON_2S$: C, 36.35; H, 6.06; N, 21.20; Found: C, 36.57; H, 6.09; N, 21.05.

N-acetyl-2-amino-1,3-propane diol.--2-amino-1,3-propane diol (4.5 gr.) was dissolved in 25 ml. of pyridine; the solution was cooled to 0°C. Acetic anhydride (5.0 gr.) was added, and the solution was allowed to stand for twenty-four hours at room temperature. The pyridine and acetic acid were removed by distillation at 50°C. at 15 mm. pressure. The residual oil was taken up in acetone, and ether was added to the solution until a faint opalescence was observed, then the solution placed in the icebox overnight. A crystalline precipitate weighing 4.3 grams was obtained. The solid, when recrystallized from acetone, melted at 87-8°C.

Analysis: Calcd. for $C_5H_{11}O_3N$: N, 10.53; Found: N, 10.23.

The N, O-diacetyl and the N,O,O'-triacetyl derivatives of 2-amino-1,3-propane diol.--In a 500 ml. round bottom flask, 18.4 grams of 2-amino-1,3-propane diol was dissolved in 100 ml. of dry pyridine. The solution was cooled at 0°C., and 40.4 grams of acetic anhydride was added slowly. The solution was allowed to stand overnight. The pyridine and acetic acid were removed by distillation at 50°C. under 15 mm. pressure. The residual oil was distilled, and the fraction boiling from 145 to 160° under 3mm. pressure was separated. A second fractionation of this oil

gave 10.4 grams of a colorless oil which boiled at 153-8°C. under 4 mm. pressure.

Analysis: Calcd. for $C_7H_{13}O_4N$: N, 8.00; Found: N, 7.52.

The triacetyl derivative can be isolated from the crude reaction mixture by taking up the mixture in acetone and precipitating the product from the solution by the addition of ether. The procedure must be repeated several times in order to obtain more material.

Analysis: Calcd. for $C_9H_{15}O_5N$: N, 6.46; Found: N, 6.00.

The reaction of N-acetyl-2-amino-1,3-propane diol with hydrogen bromide.--The monoacetyl derivative described above (13.3 gr.) dissolved in 50 ml. of dry dioxan and 1 ml. of glacial acetic acid, was placed in a bomb tube. Hydrogen bromide was then bubbled through the solution until 8.1 grams of the acid was absorbed. The tube was sealed, warmed on a steam bath for one hour, and allowed to stand overnight. The dioxan was removed under reduced pressure and the residual oil was subjected to high vacuum distillation. A thick viscous oil (14.3 gr.) boiling between 110°C. and 120°C. under 10^{-5}mm. pressure was obtained.

Analysis: Calcd. for $C_7H_{12}O_3NBr$: N, 5.88; Br, 33.58; Found: N, 6.20; Br, 38.90.

The high bromine value was due to contamination of the main product with the dibromo derivative.

N-acetyl-β-hydroxy-α-amino-S-propyl xanthate.--Potassium ethyl xanthate (6.3 gr.) was dissolved in 75 ml. of 95 per cent ethyl alcohol. The solution was filtered into a flask containing 6.0 grams of N-acetyl-γ-hydroxy-β-amino propyl bromide in 25 ml. of ethyl alcohol. Potassium bromide separated almost at once. The mixture, after standing overnight, was filtered; the filtrate was concentrated to about 15 ml. Fifty milliliters of water was added, and the solution was extracted with five 20 ml. portions of ethyl acetate. The ethyl acetate extracts were dried over anhydrous sodium sulfate, and then concentrated to about 20 ml. The concentrate, on standing overnight, deposited 0.5 grams of white crystalls. These, when re-crystallized from methyl alcohol, melted at 137-8.5°C.

Analysis: Calcd. for $C_8H_{15}O_3NS_2$: N, 5.92; S, 26.98; Found: N,5.68, S, 26.51.

N-acetyl-2-amino-1,3-propane dithiouronium dihydrobromide.--Of the product obtained by the treatment of N-acetyl-2-amino-1,

3-propane diol with hydrogen bromide, 2.4 grams was dissolved in 10 ml. of absolute alcohol; 0.8 gram of thiourea was then added. The solution was refluxed under anhydrous conditions for two hours; after forty-five minutes a solid began to form. The solution was cooled, and the crude product, which weighed 2.0 grams, was removed by filtration. The product recrystallized very slowly from 95 per cent alcohol; the recrystallized material melted at 214-5°C. with decomposition.

Analysis: Calcd. for $C_7H_{17}ON_5S_2Br_2$: N, 17.03; Br, 38.96; Found: N, 16.88; Br, 39.20.

N,O-dibenzoyl-2-amino-1,3-propane diol.--In a three-necked 500 ml. flask equipped with stirrer, dropping funnel, and condenser was placed a solution of 2-amino-1,3-propane diol, 18.2 grams, in 200 ml. of dry pyridine. The solution was cooled in an ice-bath and fifty-six grams of benzoyl chloride was added very slowly over a period of two hours. The reaction mixture was allowed to stand overnight at room temperature. The pyridine solution was poured into 200 ml. of water; after forty-five minutes the resulting mixture was filtered. The solid obtained was predominantly the tribenzoyl derivative. The filtrate was diluted with water to 1.5 liters and placed in the ice-box overnight. The crude dibenzoyl derivative separated as a yellow solid. When this solid was crystallized from aqueous ethyl alcohol, there was obtained 19.0 grams of a product which melted at 127-8°C.

Analysis: Calcd. for $C_{17}H_{17}O_4N$: C, 68.30; H,5.69; N, 4.67; Found: C, 68.39; H, 5.49; N, 4.48.

The rearrangement of N,O-dibenzoyl-2-amino-1,3-propane diol to O,O-dibenzoyl-2-amino-1,3-propane diol hydrobromide.--The N,O-dibenzoyl derivative, 1.0 gram, was dissolved in 10 ml. of dioxan, and placed in a bomb tube. Hydrogen bromide was passed into the solution until 1.0 gram had been absorbed. The tube was sealed and heated to 85°C. for three hours. The dioxan was removed under reduced pressure, the residue was dissolved in hot chloroform and filtered. Low boiling ligroin was added to the filtrate until the first sign of opalescence appeared; then the solution was placed in the ice-box. The white solid (0.1 gr.) which deposits was collected on a filter. The solid recrystallized from n-propyl alcohol melted at 196-7°C. with decomposition.

Analysis: Calcd. for $C_{17}H_{18}O_4NBr$: C, 53.73; H, 4.74; Found: C, 53.40; H, 4.83.

Further addition of ligroin to the filtrate, precipitated the starting material.

Bis-(α-amino-β-hydroxy)-propyl sulfite dihydrochloride.--2-amino-1,3-propane hydrochloride (3.5 gr.) was suspended in 20 ml. of chloroform. To this solution, which was vigorously agitated, was added a solution of 3.3 grams of thionyl chloride in 15 ml. of chloroform; the resulting mixture was kept at 50°C. for 1.5 hours. The solvent was removed by distillation under reduced pressure, and the solid residue was crystallized from absolute alcohol. There was obtained 2.7 gr. of a crystalline solid which melted at 103-4°C.

Analysis: Calcd. for $C_6H_{18}O_4NS_2Cl_2$: N, 9.30; Cl, 23.60; Found: N,9.59; Cl,24.00.

N,O-dibenzoyl-α-amino-β-hydroxy propyl chlorosulfite.--A solution of N,O-dibenzoyl-2-amino-1,3-propane diol (3.0 gr.) in 15 ml. of thionyl chloride was allowed to stand at room temperature for twenty-four hours. The solution was warmed on a steam bath for one hour, and the excess thionyl chloride removed by distillation under reduced pressure. The solid residue was recrystallized from ligroin. The product weighed 2.5 grams and melted at 94-5°C.

Analysis: Calcd. for $C_{17}H_{16}O_5NSCl$: N, 3.68; Found: N, 3.22.

This product, the chlorosulfite, was unstable; it decomposed slowly at room temperature on exposure to the atmosphere. In toluene solution, the chlorosulfite decomposed to the N,O-dibenzoyl-β-amino-γ-hydroxy propyl chloride which melted at 124-5°C.

Analysis: Calcd. for $C_{17}H_{16}O_3NCl$: Cl, 11.18; Found: Cl, 11.23.

N,O-dibenzoyl-α-amino-β-hydroxy propyl chloride.--Phosphorous pentachloride (15.0 gr.) dissolved in 150 ml. of dry chloroform was placed in a 500 ml. round-bottom flask equipped with condenser and calcium chloride drying tube. A suspension of 15.0 grams of N,O-dibenzoyl-2-amino-1,3-propane diol in 135 ml. of chloroform was added in four portions. The solution was refluxed for three hours. The excess phosphorous pentachloride, phosphorous oxychloride, and the chloroform were removed by vacuum distillation. The residual oil was taken up in 75 ml. of chloroform, and hydrogen chloride was passed through the solution for one hour. After the solution had stood overnight, the chloroform was removed under reduced pressure; the residual oil was taken up in a minimum amount of absolute alcohol. When the solution was cooled, it solidified.

The solid, when recrystallized from absolute alcohol weighed 10.3 grams. This compound was identical with that obtained by the decomposition of the chlorosulfite described above.

N,O-dibenzoyl-α-amino-β-hydroxy propyl thiouronium chloride.--The chloride described above (10 gr.) and thiourea (2.5 gr.) were added to 50 ml. of n-propyl alcohol. The solution was refluxed for five hours and then concentrated to one-third of its original volume. Ether was added until an oily precipitate formed; then the flask placed in the icebox overnight. The solid precipitate was collected on a filter and crystallized from an alcohol-ether mixture thus 7.3 grams of the thiouronium derivative was obtained. The compound melted at 159-61°C.
Analysis: Calcd. for $C_{18}H_{20}N_3SCl$: C, 54.80; H,5.09; N, 10.68;
Found: C,54.84; H, 5.39; N, 10.86.

Bis(N-benzoyl-α-amino-β-hydroxy) propyl disulfide.--The thiouronium salt (7.0 gr.) was suspended in a solution consisting of 20 ml. of water, 20 ml. of alcohol, and 1.1 grams of potassium hydroxide. The mixture was warmed on a steam bath for three hours. Then the solution was cooled, 2 ml. of 2 per cent ferric chloride solution was added, and air was bubbled through the mixture until only a faint sulfhydryl test was obtained with sodium nitroprusside. An equal volume of water was added and the solution was placed in the icebox overnight. A gummy solid separated. This was collected and crystallized from 95 per cent ethyl alcohol. The yield was 2.3 grams of crystalline material which melted from 145-7°C.
Analysis: Calcd. for $C_{20}H_{24}O_4N_2S$: C, 57.20; H, 5.75; N, 6.67;
Found: C, 57.52; H, 5.82; N, 6.51.

Bis(N,O-dibenzoyl-α-amino-β-hydroxy) propyl disulfide.--N,O-dibenzoyl-α-amino-β-hydroxy propyl thiouronium chloride, 7.0 grams, was suspended in a solution consisting of 35 ml. of 95 per cent ethyl alcohol and 3 ml. of water containing 1.1 grams of potassium hydroxide. The solution was warmed on a water bath for three hours. Two milliliters of 2 per cent solution of ferric chloride was added to the cooled mixture; the addition of the ferric chloride produced a transient purple coloration. When air was bubbled through the solution for several hours, a white solid was formed. This material was insoluble in water and only slightly soluble in alcohol. The yield of this product was 4.2 grams; the substance, after crystallization from acetone, melted at 190-1°C.

Analysis: Calcd. for $(C_{17}H_{16}O_3NS)_2$: C, 65.00; H,5.09; Found: C, 64.74; H, 5.14.

Bis(a-amino-B-hydroxy) propyl disulfide dihydrochloride, cystinol hydrochloride.--The mono- or dibenzoyl derivative of bis (α-amino-β-hydroxy) propyl disulfide (2 gr.) was placed in a bomb tube. Absolute ethyl alcohol, 50 ml., was added and hydrogen chloride was passed through the solution for twenty minutes. The tube was then sealed and heated on a water bath at 50°C. for four to five hours. The alcohol was removed from the product by evaporation in vacuo, and the residue was recrystallized from an acetone-absolute alcohol mixture. There was abtained 0.3 to 0.5 grams of a colorless, water soluble solid which melted at 178-80°C. Analysis: Calcd. for $(C_3H_9ONSCl)_2$: C, 25.25; H, 6.32; N, 9.83; Found: C, 25.49; H, 6.35; N, 9.47.

SUMMARY

The clinical and pharmacological data on the oxytocic principle of the posterior lobe of the pituitary gland are reviewed. The synthesis of cystinol hydrochloride, the amino alcohol analogous to cystine, from 2-amino-1,3-propane diol is described. The chemical properties and of 2-amino-1, 3-propane diol and of some of its derivatives are also described.

Cystinol at a concentration of 1 part in 85,000 causes weak contractions in the isolated uterus of a virgin guinea pig.

Reactions Involved in the Synthesis of Cystinol Hydrochloride